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MONOSYNAPTIC EPSPs ELICITED BY SINGLE INTERNEURONES AND SPINDLE AFFERENTS IN TRIGEMINAL MOTONEURONES OF ANAESTHETIZED RATS

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SUMMARY

1. Our aim has been to quantify the monosynaptic connections of trigeminal interneurones and spindle afferents onto jaw-elevator motoneurones as a step towards identifying common features in organization of monosynaptic inputs onto motoneurones. We have used the intracellular variant of the spike-triggered averaging method to examine the connections of single identified trigeminal interneurones and jaw-elevator muscle spindle afferents onto single jaw-elevator motoneurones. The interneurones examined lay in the region immediately caudal to the trigeminal motor nucleus. The experiments were performed on rats anaesthetized with pentobarbitone, paralysed and artificially ventilated.

2. Ten EPSPs and eight IPSPs were obtained from examining the connections of seventeen interneurones to thirty-six motoneurones, suggesting a functional connectivity of 50% for individual interneurones onto elevator motoneurones. Fourteen EPSPs were obtained from examining the connections of thirteen spindle afferents onto twenty-seven motoneurones, giving a functional connectivity of 52% for individual spindle afferents onto elevator motoneurones. The amplitudes of the EPSPs elicited by interneurones ranged from 7–48 μV (mean = 17, S.D. = 12·5, $n = 10$) and from 7 to 289 μV (mean = 64, S.D. = 76·0, $n = 14$) for the spindle-mediated EPSPs; the difference in the two means was not significant ($P = 0{\cdot}07$).

3. However, the amplitude of averaged responses obtained by signal averaging methods are dependent on the assumption that the postsynaptic response occurs following every impulse in the presynaptic neurone. We therefore estimated the percentage of sweeps which contained EPSPs triggered by the presynaptic neurone under study. In essence the method used consisted of visual inspection of the individual sweeps comprising an average in order to assess the occurrence of EPSPs within six separate time windows, each of duration $\pm 0{\cdot}3$ ms. Five windows were placed at randomly selected times on average and were used to provide an estimate of the frequency of occurrence of randomly triggered EPSPs. The sixth window was centred on the start of the averaged EPSP and the frequency of occurrence of randomly triggered EPSPs was subtracted from the frequency of occurrence of EPSPs in this window to produce an estimate of the incidence of EPSPs triggered by the presynaptic neurone under study.

4. Values of the incidence of occurrence of EPSPs triggered by the presynaptic

neurones ranged from 4·3 to 92% for the fifteen averaged EPSPs which could be analysed in this manner (two elicited by interneurones and thirteen by spindle afferents). There was a statistically significant relationship ($r = 0{\cdot}98$; $P < 0{\cdot}001$) between the incidence of occurrence of EPSPs and the amplitude of the averaged EPSP. The relationship took the form of a logistic sigmoid curve and two conclusions were drawn from this. First, for averaged EPSPs of less than approximately 150 μV amplitude, differences in amplitude of the averaged EPSP elicited by different presynaptic neurones can be accounted for primarily by differences in the incidence of failures of transmission. Second, for averaged EPSPs greater than 150 μV in amplitude, differences in the amplitude of averaged EPSPs elicited by different presynaptic neurones may primarily be due to differences in either the number of active release sites or the quantal size.

INTRODUCTION

Our current understanding of the rules governing the organization of monosynaptic connections onto motoneurones, and of the events occurring at such synapses, are based largely on data obtained on the connections of muscle spindle afferents onto motoneurones. The most intensively studied connection has been that of hindlimb spindle primaries onto hindlimb motoneurones (Mendell & Henneman, 1971; for review see Henneman & Mendell, 1981), but the connections of jaw-elevator (Appenteng, O'Donovan, Somjen, Stephens & Taylor, 1978), intercostal (Kirkwood & Sears, 1982), and neck muscle spindle afferents (Keirstead & Rose, 1988) have also been studied onto their homonymous motoneurones. Other monosynaptic excitatory connections that have been studied include the connections of bulbospinal neurones onto intercostal motoneurones (Davies, Kirkwood & Sears, 1985), and corticospinal fibres onto motoneurones (Asanuma, Zarzecki, Jankowska, Hongo & Marcus, 1979). However, these connections have in general been studied less extensively than those of spindle afferents onto motoneurones and so as a result there is uncertainty as to whether data obtained on connections of spindle afferents are relevant to other monosynaptic inputs onto motoneurones. The question is one that cannot be resolved until there is comparable detailed electrophysiological and morphological data on other monosynaptic excitatory connections onto motoneurones.

However, the stumbling block has been in identifying other convenient sources of monosynaptic excitatory inputs onto motoneurones. Input from last-order interneurones (i.e. interneurones with monosynaptic connections onto motoneurones) would be an obvious additional class of input to study but most last-order interneurones identified have been ones with inhibitory actions on motoneurones and so it has not been readily possible to compare data on these connections to the data on the monosynaptic excitatory connections of muscle spindle afferents onto motoneurones.

Indeed only four groups of excitatory last-order interneurones have been identified, three located in the spinal cord and one in the region immediately caudal to the trigeminal motor nucleus. Of the spinal interneurones, one group are located in the C3–C4 spinal segments and project to forelimb motoneurones (for reviews see Lundberg, 1979, and Baldissera, Hultborn & Illert, 1981), another in the C7–C8 segments and project to fore-limb motoneurones (Hongo, Kitazawa, Ohki & Xi,

1989), and another in the L3–L4 segments and project to hindlimb motoneurones (Cavallari, Edgley & Jankowska, 1987). However, there have been no reports of the actions of either single C3–C4 or single L3–L4 interneurones on motoneurones, and only a preliminary report of the actions of three C7–C8 interneurones on single forelimb motoneurones (Hongo *et al.* 1989).

We therefore attempted to address this issue by quantifying the functional connectivity and amplitude of the averaged EPSPs elicited by single trigeminal interneurones and spindle afferents in single elevator motoneurones. The hope was that a comparison of data obtained under the same experimental conditions from the same motor system would facilitate the identification of common features of organization. Our conclusions are that individual spindle afferents and interneurones connect to similar numbers of motoneurones and elicit EPSPs of similar amplitude. There is a high incidence of failures of transmission at the synapses of both interneurones and spindle afferents onto elevator motoneurones and as a consequence both the true amplitude and functional connectivity of individual spindle afferents and interneurones will tend to be underestimated. Preliminary accounts of this work have been communicated to the Physiological Society (Appenteng, Curtis & Moore, 1990, Grimwood & Appenteng, 1991*b*).

METHODS

Surgical preparation. This was essentially as described earlier by Appenteng, Conyers & Moore (1989). In brief, thirty rats in the weight range 200–250 gm were initially anaesthetized with a mixture of halothane in oxygen. A femoral venous catheter was inserted and further anaesthesia maintained by I.V. infusions of pentobarbitone (initial dose = 60 mg/kg I.V.). The trachea was cannulated and blood pressure monitored by a cannula in the femoral artery. The left masseter nerve was exposed in-continuity and a pair of silver wires placed around the nerve to allow electrical stimulation. Animals were then transferred to a stereotaxic holder, their heads held as described by Appenteng *et al.* (1989), paralysed with gallamine triethiodide and artificially ventilated for the duration of the experiment. A bilateral pneumothorax was performed and end-tidal carbon dioxide levels monitored. Supplementary doses of pentobarbitone (1·2 mg I.V.) were given as necessary throughout the experiment so as to maintain a continuous deep level of anaesthesia at all times. The criterion used to assess the depth of anaesthesia was that a noxious paw pinch should elicit no change in blood pressure and under these conditions there was no flexion withdrawal reflex in the unparalysed animal.

Electrodes. Extracellular recordings from single neurones were made with glass microelectrodes filled with a 1 M solution of DL-homocysteic acid (DLH; pH = 8·0). Intracellular recordings were made from elevator motoneurones using electrodes filled with either 2 or 4 M-potassium acetate, and bevelled to resistances of 7–11 MΩ.

Protocol. This was essentially as described by Appenteng *et al.* (1989) and was shaped by the need to position the tips of two electrodes within 700 μm of each other in structures some 8 mm below the surface of the cerebral cortex. In brief an electrode filled with DLH was first placed in position by locating the middle of the masseter motoneurone pool, signalled by the presence of an antidromic field of amplitude 1·5–2·0 mV. The electrode was then withdrawn and positioned 400–500 μm more caudally so as to place it immediately caudal to the motor nucleus. We have previously shown that the region starting at the caudal border of the motor nucleus and extending for some 400 μm further caudally contains a population of interneurones which make synaptic connection within the elevator motoneurone pool (Appenteng & Girdlestone, 1987; Appenteng *et al.* 1989). Single units were isolated with the DLH electrode and their projection to the motor nucleus studied by constructing perispike averages. Neurones that lay outside the motor nucleus or the mesencephalic nucleus (areas known to contain motoneurones and afferents respectively) were assumed to be interneurones for the purposes of this study. Thus on this basis any somatic recordings obtained from outside these areas must by definition be from interneurones. The search strategy used was to track with a continuous low (less than 0·2 nA) ejection of DLH and to

electrically stimulate the masseter nerve at 1 s intervals. Iontophoretic application of DLH was then used to distinguish between somatic and axonal recordings. We have previously reported that iontophoretic application of DLH can be used to distinguish somatic and axonal recordings as the former show an increased firing to DLH application whereas the latter show no change in firing (see Fig. 1 of Appenteng *et al.* 1989). Axonal recordings were ignored as these could have originated from fibres of passage. Somatic recordings were then further examined to determine the pattern of inputs onto them using both natural (non-nociceptive) stimulation applied to the mandibular and maxillary areas and also electrical stimulation of the masseter nerve.

Recordings were also made from spindle afferent axons within the mesencephalic tract. These were identified by the criteria of increased firing on gentle muscle probing and increased firing on jaw opening (see Appenteng, Donga & Williams, 1985). Most spindle afferents generally fired tonically, with the jaw unsupported, at intervals of between 30–70 ms. However, on a few occasions tonic firing at these rates had to be induced by hanging a small weight onto the jaw to increase the degree of opening (muscle stretch).

Having isolated either an identified single interneurone or spindle afferent, a potassium acetate-filled electrode was inserted into the motor nucleus and attempts then made to obtain intracellular penetrations of elevator motoneurones with it. Masseter and masseter synergist motoneurones were respectively identified by their antidromic activation or synaptic activation following electrical stimulation of the masseter nerve (see Moore & Appenteng, 1990). A 5 min sealing-in period was then generally allowed following penetration and the motoneurone then only accepted for inclusion in the study if the membrane potential (V_m), which was continuously monitored, was at least 45 mV at the start of recording and then did not fall below 39 mV throughout the period of averaging.

Data acquisition. All data were recorded on-line using a C.E.D. 1401 interface (Cambridge Electronic Design). The signals recorded were; (1) the individual sweeps of a low gain signal (filter settings = DC, −5 kHz; poststimulus mode) of the motoneurone response to electrical stimulation of the masseter nerve, (2) a high gain filtered (0·5 Hz to 5 kHz) signal of the synaptic noise (perispike mode), (3) an arbitrarily filtered signal of the spikes of the triggering neurone (perispike mode), and (4) where appropriate, the response of the triggering neurone to electrical stimulation of the masseter nerve (poststimulus mode). The sampling rate was set at 40 kHz for both the perispike and poststimulus modes. In the perispike mode, sweeps were rejected from the average if the high gain intracellular signal from the motoneurones exceeded plus or minus 5 mV. The sweep duration in the perispike mode was set at 22·5 ms for most experiments (7·5 ms pre-trigger and 15 ms post-trigger).

In the last eleven experiments of the series, the individual sweeps used to construct the intracellular perispike averages were saved on disk and subsequently analysed in the way described by Sayer, Redman & Andersen (1989; see also Jack, Redman & Wong, 1981). The starting point of the analysis is that in a perispike average, points occurring before the onset of the triggering spike can be regarded as constituting the noise-only portion of the signal, whereas points occurring after the trigger spike are composed of signal (i.e. EPSP) plus noise. The assumption that the prespike period is 'noise only' is of course only valid if spikes from the triggering neurone do not occur in the prespike period. To guard against this, we routinely examined the individual sweeps recorded from the triggering neurone in the perispike average and were able to verify the absence of additional spikes from the triggering neurone in the prespike period. Similarly, we were able to verify, in the case of spindle afferents, that additional triggering spikes did not occur in the postspike period. However, some interneurones fired additional spikes during the postspike period but this did not include any subjected to an analysis of individual sweeps. The amplitude of the noise-only and noise + EPSP components of the trace were determined for each of the individual sweeps comprising an average in the way illustrated in Fig. 1. The peak of the EPSP was identified and the mean voltage in the region encompassing the peak and three data points each side of it (i.e. three times 0·025 ms) determined ('2' of Fig. 1). The duration of this window set the duration over which all other voltages were then measured. In general the window ranged from 0·25–0·4 ms for the sample of EPSPs analysed. A region immediately preceding the start of the EPSP ('1' in Fig. 1) was identified and the amplitude of the EPSP + noise component derived by subtraction of the two voltages measured on individual sweeps. The amplitude of the noise-only component was estimated by subtraction of mean voltages measured at '2'' and '1'' of Fig. 1. The bar labelled '1'' in Fig. 1 was set to begin at the start of the sweep, and the temporal separation between '1'' and '2'' set to equal that between '1' and '2' (see Fig. 1). Following Sayer *et al.* (1989), we used Fisher's z

approximation to the F distribution (where $z = \sqrt{\text{number of sweeps}} \times \log$ (standard deviation of noise/standard deviation of noise plus EPSP) to test the null hypothesis that the two histograms were drawn from distributions having the same standard deviation.

Electrode tracts were identified using standard histological techniques after completion of experiments.

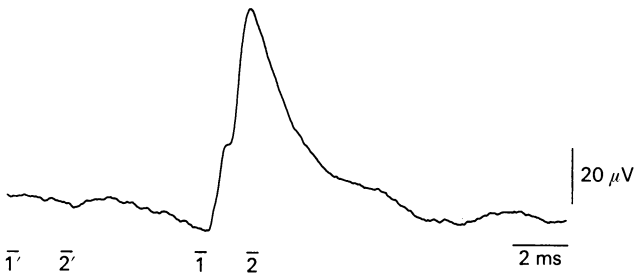

Fig. 1. Illustration of the procedure used to determine the amplitude of the noise-only and noise + EPSP components. The amplitude of the EPSP + noise component of the signal was obtained by subtraction of the voltages at '2' and '1', and the amplitude of the noise-only component of the signal obtained by subtraction of the voltages at '2′' and '1′'. See text for additional details. EPSP shown is EPSP 3 in Table 1.

RESULTS

Averaged EPSPs elicited by spindle afferents and interneurones

Figure 2 shows the averaged intracellular responses elicited by a spindle afferent (Fig. 2*Aa–d*) and an interneurone (Fig. 2*Ba–d*), each in four different elevator motoneurones. The responses were all recorded in the same experiment during the course of a single electrode track through the motor nucleus. Both the afferent and interneurone project to only one of the four motoneurones to which they were tested and this finding of a limited connectivity was a consistent feature of our data.

Altogether, we have examined the connections between 122 pairs of interneurones and motoneurones (78 interneurones and 112 motoneurones) and obtained EPSPs in ten cases and IPSPs in eight others. On one occasion we were able to examine the connections of six interneurones to the same motoneurone, on two others the connections of three interneurones to the same motoneurone, and on another two occasions the connections of two interneurones to the same motoneurone. However, the usual situation was that the connections of a single interneurone were examined to one motoneurone ($n = 48$ interneurones; three EPSPs elicited), but nineteen interneurones each had their connections tested to two motoneurones (three EPSPs and eight IPSPs), seven interneurones to three motoneurones each (one EPSP), and four interneurones to four motoneurones each (three EPSPs). The connectivity of interneurones onto elevator motoneurones can be estimated by considering just those interneurones which elicited EPSPs or IPSPs in elevator motoneurones. The ten EPSPs and eight IPSPs were obtained by examining the connections of seventeen interneurones onto thirty-six motoneurones, giving a connectivity of 50% for the sample, or a connectivity of 47% if interneurones examined to only a single motoneurone are excluded (sixteen synaptic events from fourteen interneurones and thirty-four motoneurones).

We have also examined the connections between seventy-six pairs of spindle afferents and motoneurones (fifty-one afferents to seventy-four motoneurones) and obtained EPSPs in fourteen cases. On two occasions the connections of two afferents were studied to the same motoneurone. Thirty-four afferents each had their

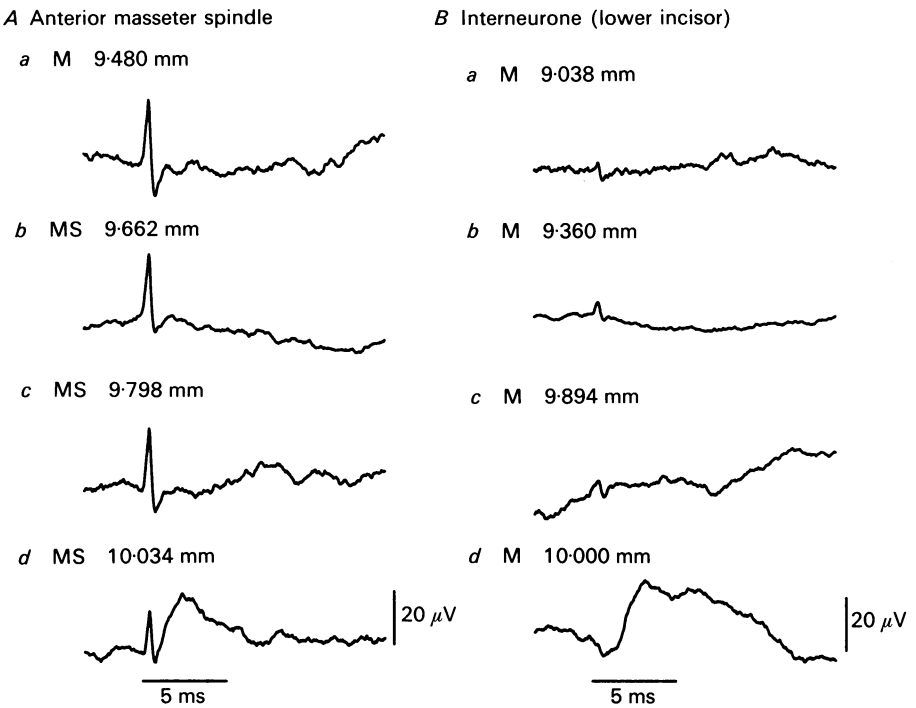

Fig. 2. Averages obtained when triggering off a masseter spindle afferent (*Aa–b*) and an interneurone (*Ba–b*) which was activated by pressure to the lower incisor. Both the afferent and interneurone were studied to four motoneurones. The depths of the motoneurones are indicated by the numerals above each trace, and the motoneurone types indicated by an M for masseter, and MS for masseter synergists. The afferent and interneurone each elicit an EPSP in only one of the four motoneurones (A4 and B4), though an apparent 'presynaptic spike' is present in all averages. Numbers of sweeps used: *Aa* = 500, *Ab* = 1297, *Ac* = 500, *Ad* = 603, *Ba* = 520, *Bb* = 3037, *Bc* = 1000, and *Bd* = 1380. Spindle EPSP = EPSP 2 in Table 1 and interneurone EPSP = EPSP 14. Start of time bar marks time zero in this and all subsequent figures.

connections examined to a single motoneurone only (five EPSPs), but eleven afferents had their connections examined to two motoneurones each (five EPSPs), four afferents to three motoneurones each (two EPSPs), and two afferents to four motoneurones each (two EPSPs). The connectivity for the total sample was estimated as 52% (fourteen EPSPs, thirteen afferents, and twenty-seven motoneurones), or 41% if afferents examined to only a single motoneurone are excluded (nine EPSPs, eight afferents, and twenty-two motoneurones). On four occasions we were able to examine the connections of a masseter spindle afferent to two masseter motoneurones and on another the connections of a masseter afferent to three masseter

motoneurones. No EPSPs were obtained from these nine pairs. However, two EPSPs were obtained when the connections of three temporalis spindle afferents were each examined to two masseter motoneurones. Although the sample sizes are admittedly small, there is at least a hint at the possibility that the homonymous projection of

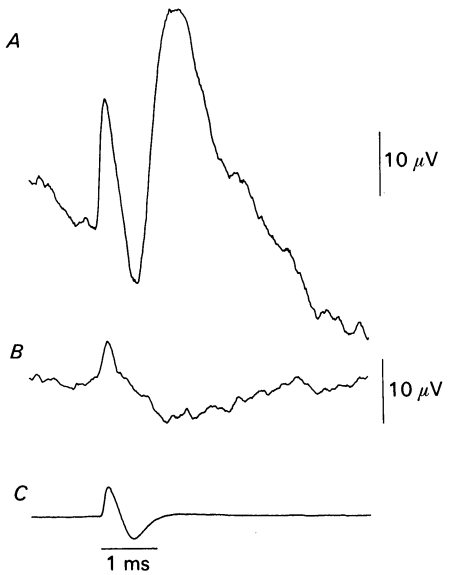

Fig. 3. Intracellular (*A*; EPSP 7 in Table 1) and extracellular (*B*) averages obtained when triggering off a masseter spindle afferent (spike average shown in *C*). The intracellular average was obtained in a masseter motoneurone (492 sweeps used) and the extracellular average obtained immediately extracellular to the same motoneurone (1000 sweeps used). Note the short latency difference between the onset of the extracellular field in *B* and the averaged EPSP in *A*. Note also that the positive peak of the triggering spike (*C*; average of sixty sweeps) coincides with the positive peak of the apparent 'presynaptic' spikes in *A* and *B*, suggesting that the 'presynaptic' spikes may be induced by coupling between the two electrodes.

masseter spindle afferents may be lower than that of some synergist afferents (i.e. temporalis) onto masseter motoneurones.

A prominent feature in Fig. 2 is the triphasic spike seen around time zero in each average. We initially assumed that these represented presynaptic or terminal spikes (Appenteng *et al.* 1989) but subsequently found that the positive peak of these 'presynaptic' spikes often coincided with the positive peak of the spike recorded from the triggering neurone, making it difficult to be certain if the former really were 'presynaptic' spikes or in part an artifact caused by coupling between the two recording electrodes (Fig. 3; see also Fig. 1 of Hongo *et al.* 1989). The uncertainty as to the origin of the 'presynaptic' spike meant that we were unable to systematically obtain separate estimates of conduction time to the motor nucleus and synaptic delay of EPSPs. Instead we determined the overall latency from the onset of the triggering spike to the onset of the EPSP. The mean latency of EPSPs elicited by spindle afferents was 1·0 ms (range = 0·73–1·87 ms, S.D. = 0·31, $n = 14$) but latency

values for spindle afferents are somewhat ambiguous as an action potential from the periphery would arrive first at the motor nucleus before proceeding to the sites caudal to the motor nucleus where the afferent activity was recorded (Appenteng *et al.* 1985). Therefore, the apparent conduction time is dependent on the relative conduction velocities along the axonal branch to the recording site and on the conduction velocity along the terminal collaterals in the motor nucleus (Appenteng *et al.* 1978). Nevertheless our assumption was that these EPSPs were monosynaptically generated. On four occasions we were able to record EPSPs in motoneurones from spindle afferents, and subsequently clear unitary extracellular fields from the same afferents on withdrawing the electrode from the motoneurone (Fig. 3). The latencies of the averaged extracellular fields and the averaged EPSPs differed by 0·1, 0·22, 0·30 and 0·67 ms, giving a mean difference of 0·32 ms. The differences in latency are best regarded as only approximate as the extracellular fields did not have a distinct onset (Fig. 3) and so it proved difficult to obtain reliable latency estimates from them. We have previously shown that the extracellular fields elicited by spindle afferents in the motor nucleus have a monosynaptic origin (Appenteng *et al.* 1989), and therefore the short latency differences between the onset of the extracellular fields and EPSPs are sufficiently short as to suggest that the EPSPs also have a monosynaptic origin.

We did not record any clear unitary extracellular fields from interneurones which generated EPSPs in motoneurones, but the earlier data of Appenteng *et al.* (1989) can be used to obtain an estimate of the average latency to the onset of the extracellular field. The mean conduction time into the motor nucleus was reported as 0·35 ms (range = 0·06–0·96 ms, S.D. = 0·22, $n = 46$), and the mean synaptic delay as 0·43 ms (range = 0·23–0·96, S.D. = 0·1, $n = 46$). This gives a mean total latency of 0·78 ms for the onset of unitary extracellular fields in the motor nucleus following the onset of an interneurone spike. The data from the present study give a mean latency of 1·1 ms to the onset of the EPSPs elicited by interneurones (range = 0·54–1·25 ms, S.D. = 0·41, $n = 6$). The difference of 0·32 ms is sufficiently short as to suggest that interneurone EPSPs are also compatible with a monosynaptic origin.

Six of the eight IPSPs elicited by interneurones occurred at latencies of 2·0–3·0 ms, and the remaining two at latencies of 0·62 and 0·70 ms. The values below 1·25 ms would be compatible with monosynaptic transmission whereas values of 2·0 ms and above may not be. The caveat is that we assume that interneurones generating IPSPs have similar axonal conduction velocities and synaptic delays as those generating EPSPs. The amplitude of IPSPs ranged from 7·8–33 μV (mean = 15·1, S.D. = 7·5, $n = 8$).

Most interneurones examined (41/78) were activated by non-noxious input from intraoral structures, with thirty-one being activated solely by intraoral input, eight by both intraoral and cutaneous inputs, and two by both muscle and intraoral inputs. A further fifteen interneurones were activated by cutaneous input only, three by input from the masseter muscle, and four by jaw opening (muscle stretch). Receptive fields could not be found for fifteen interneurones. Five of the eight IPSPs were obtained from interneurones activated solely by intraoral input, one from an interneurone activated by both intraoral and cutaneous input, one from an interneurone activated by cutaneous input only, and one from an interneurone with

an unidentified receptive field. Two of the ten EPSPs were elicited by interneurones which were activated solely by intraoral input, three by interneurones activated by cutaneous and intraoral inputs, one by an interneurone activated by both intraoral and muscle afferent input, one by interneurone activated by cutaneous input only, one by an interneurone activated by muscle stretch, and two by interneurones with unidentified receptive fields. A significant feature revealed here is that interneurones activated by input from intraoral structures can elicit either EPSPs or IPSPs in jaw-elevator motoneurones and so provides evidence that input from a single modality can contribute to the control of motoneurone behaviour by virtue of activation of both positive and negative feedback loops (see Appenteng *et al.* 1989 and Appenteng, 1991).

Twenty-eight of the fifty-one spindle afferents from which recordings were made were localized to the masseter muscle, seventeen to the temporalis muscle, but the location of six afferents could not be identified with any certainty to a specific muscle. We did not attempt to separately identify spindles as primaries or secondaries. The only certain means of achieving such a distinction involves selective activation of intrafusal muscle fibres, but given that the animals were continuously paralysed with gallamine triethiodide, any such attempt would have foundered against the fact that the paralysing agent itself exerts differential effects on intrafusal bag and chain fibres (see chap. 5 of Matthews, 1972). There are no differences in conduction velocity of jaw-elevator muscle spindle primaries and secondaries and so we would assume that our sample of spindles would presumably be composed of a random admixture of primaries and secondaries (for review see Appenteng, 1990).

The amplitudes of EPSPs elicited by spindle afferents ranged from 7 to 289 μV (mean = 64 μV, S.D. = 76·0, n = 14), and from 7 to 48 μV (mean = 17 μV, S.D. = 12·5, n = 10) for interneurones (Fig. 4); the difference between the two means was not significant (P = 0·07). The average membrane potentials of motoneurones to which the connections of spindle afferents were examined ranged from 40 to 86 mV during the periods of averaging (mean = 48, S.D. = 9·3, n = 74). The average V_m in motoneurones in which EPSPs from spindle afferents were obtained was 45 mV (range = 40–64, S.D. = 6·3, n = 14), and 49 mV (range = 40–86, S.D. = 9·8, n = 60) in motoneurones where no EPSPs were obtained. The difference in the two means was not significant (P = 0·20). There was also no statistical difference (P = 0·84) in the average membrane potentials, during periods of averaging, for motoneurones in which EPSPs or IPSPs were obtained from interneurones (mean = 52 mV, S.D. = 11·2, range = 39–80, n = 18), and motoneurones in which no responses were obtained from interneurones (mean = 54 mV, S.D. = 11·6, range = 39–94, n = 94). EPSP amplitude was not related to V_m (P = 0·18), and the same held true even if spindle-mediated EPSPs were considered separately from interneuronally mediated EPSPs ($P > 0·1$ in both cases).

The data given above, together with a description of the time course of an average EPSP, the estimated connectivity of individual spindle afferents and interneurones (52 % and 50 % respectively), and an estimate of their numbers, can be used to derive an estimate of the nominal steady depolarization that would be produced in elevator motoneurones by these two inputs.

The approach has been used by Tuck (reported in Harrison & Taylor, 1981), Harrison & Taylor (1981), and Munson, Fleshman & Sypert (1982), and one of the chief assumptions underlying it is that of linear summation of EPSPs. Estimates of the numbers of interneurones in the area immediately caudal to the motor nucleus

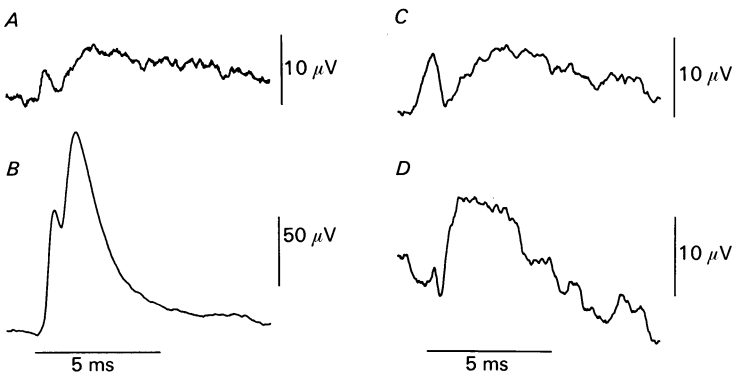

Fig. 4. Examples of EPSPs elicited by spindle afferents (*A* and *B*) and interneurones (*C* and *D*). EPSPs elicited by: *A*, masseter spindle afferent in a masseter synergist motoneurone (1081 sweeps, EPSP 9 in Table 1); *B*, anterior temporalis spindle afferent in masseter synergist motoneurone (703 sweeps, EPSP 1); *C*, by an unidentified interneurone in a masseter synergist motoneurone (900 sweeps, EPSP 15); *D*, by an interneurone, activated by input from intraoral afferents, in a masseter synergist motoneurone (512 sweeps, EPSP not listed in Table 1).

have ranged from 146–167 ($n = 3$ rats; Appenteng & Girdlestone, 1987), and if we assume that the average EPSP has a time course similar to that of the EPSP in Fig. 4*D*, then if 150 interneurones fired at a steady rate of 100 impulses/s they would generate EPSPs separated by intervals of 0·133 ms (i.e. $1/(100 \times 150 \times 0{\cdot}50)$) and this would result in a steady depolarization of 405 μV. There are no estimates of the number of masseter spindle afferents in the rat but Gottlieb, Taylor & Bosley (1984) labelled the somata of 215 spindle afferents (i.e. both primaries and secondaries) after application of horseradish peroxidase to the cut masseter nerve. A branch of this nerve supplies the anterior temporalis muscle which together with the masseter contains the bulk of elevator spindle afferents. If we assume that there are approximately 200 masseter spindle afferents, that each projects to 52% of elevator motoneurones, and that each elicits an EPSP of 64 μV, then if the afferents fired at a steady rate of 100 impulses/s they would elicit a depolarization of 2002 μV in elevator motoneurones.

Analysis of individual sweeps comprising intracellular-spike triggered average data

The estimates of the nominal steady depolarization are critically dependent on the reliability of the estimates of functional connectivity and mean EPSP amplitude. One factor which could result in an underestimation of both mean EPSP amplitude and functional connectivity would be if the synapses of spindle afferents and interneurones onto motoneurones were subject to a tonic inhibition. Inhibition at single synapses could, among other possibilities, result in a high incidence of failures

in transmission, and/or a bias towards the release of smaller quanta or the appearance of smaller quantal events (Jack *et al.* 1981; Kullman, Martin & Redman, 1989; Redman, 1990). We initially set out to attempt a distinction between these two possibilities.

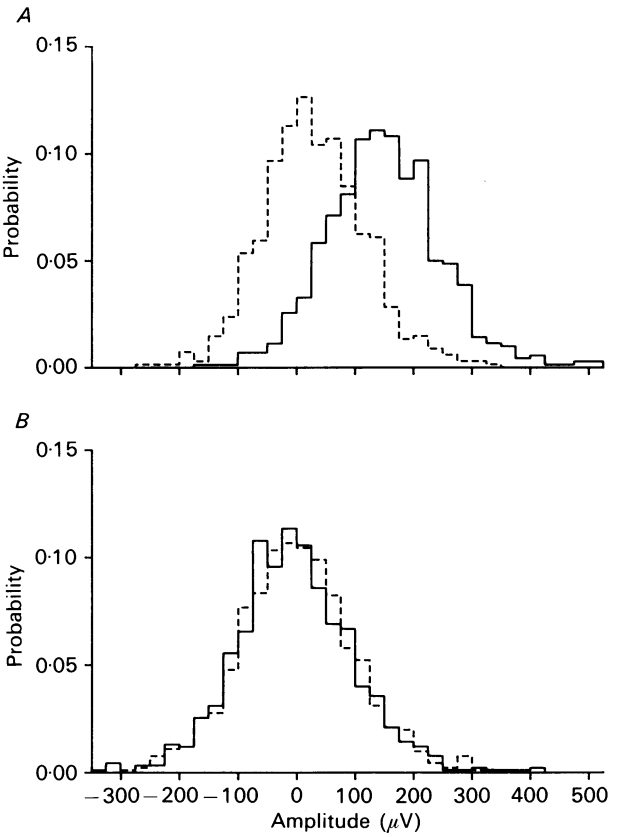

Fig. 5. Amplitude histograms of noise-only (dashed line) and noise + EPSP (continuous line) portions of responses obtained from a spindle afferent (*A*: same unit as in Fig. 4*B*) and an interneurone (*B*: same unit as in Fig. 4*C*). The afferent elicited an average EPSP of 154 μV and the interneurone an EPSP of 7·0 μV. Note the similarity in the standard deviations of the noise-only and noise + EPSP portions in both *A* and *B*.

Figure 5 shows examples of the amplitude histograms, determined as illustrated in Fig. 1, for EPSPs elicited by two presynaptic neurones in two motoneurones. The pair of neurones examined in Fig. 5*A* produced the second largest averaged EPSP obtained in this study (154 μV) whereas the pair in Fig. 5*B* produced the second smallest EPSP (7 μV) in our sample. However, in each case the standard deviation of the noise-only portion of the signal was virtually identical to that of the EPSP + noise portion of the signal and the values of z, calculated as in the Methods, were not significantly different ($P > 0{\cdot}1$ in Fig. 5*A* and *B*). Values of z obtained for all of the EPSPs examined were not significant ($P > 0{\cdot}076$ for all units; see Table 1).

Therefore, for all the EPSPs analysed, there is either no quantal variability and no fluctuations in the release of transmitter from trial to trial, or alternatively any such variations may be masked by the noise.

However, Sayer *et al.* (1989) have themselves emphasized that the above test of significance is critically dependent on the level of noise, with the chief contributions

TABLE 1. Properties of EPSPs

			Averaged EPSP			Incidence of EPSPs		
EPSP no.	Unit type	No. of sweeps	EPSP ampl.	σ_n	σ_e	Triggered (%)	Random (%)	Corrected (%)
1	Spindle	703	154·0	140·61	97·96	97	5·5	91·5
2		603	21·5	100·07	96·20	25·5	4·0	21·5
3		2026	80·4	133·69	134·87	52	6·3	45·7
4		980	19·0	139·70	148·04	14·6	6	8·6
5		350	63·0	123·01	116·65	63·2	11·6	51·6
6		367	9·0	84·05	95·21	12·8	8·5	4·3
7		492	42·4	111·91	120·05	35·6	4·6	31
8		552	11·3	124·52	122·86	10·1	3·3	6·8
9		1081	7·0	80·31	79·90	11·3	5·38	5·92
10		314	289	159·9	162·4	98·1	6·25	91·85
11		360	32·5	84·5	90·2	36·4	5·4	31
12		563	79	92·5	88·0	70	5·7	64·3
13		188	26·1	72·2	69·8	28·7	8·3	20·4
14	Interneurone	1380	26·1	427·95	408·76	16	3·06	12·94
15		900	7·0	102·45	98·02	9·7	3·1	6·6

Abbreviations: σ, standard deviation; n, noise, e, EPSP + noise. Triggered = uncorrected incidence of EPSPs in window centred on start of averaged EPSP.

to the noise being electrode noise and synaptic noise. We used electrodes with the minimum tip resistance compatible with obtaining penetrations in elevator motoneurones (soma diameter = 25 μm), and maintained the animals deeply anaesthetized so as to reduce the level of spontaneous synaptic activity. Therefore, there were few other practical measures open to us for further reducing the noise. We reasoned that a high incidence of failures of transmission could account for the masking in Fig. 5 as, depending on the incidence of failures, the EPSP + noise portion of the trace could simply consist essentially of noise and no signal on most sweeps (Fig. 5). We assessed the incidence of failures in transmission by visual inspection of the individual traces comprising an average. Essential to the analysis was an estimate of the range of variability in synaptic delay which would be expected at synapses on trigeminal motoneurones. We used, as our source for the estimation of this variability, the data of Appenteng *et al.* (1989) on syaptic delay at synapses onto trigeminal motoneurones measured using the extracellular variant of the spike-triggered averaging method. The standard deviation about the mean synaptic delay for a sample of twelve spindle afferents and forty-five interneurones was 0·11 ms (mean = 0·42). We assumed variations of similar magnitude could also occur in the synaptic delay of intracellularly recorded responses, and also that individual responses occurring with synaptic delays of more than $\pm$0·28 ms would be unlikely to be triggered by the same presynaptic neurone as this would be associated with a

probability of less than 1 %. The nearest interval we could resolve with our sampling rate of 40 kHz was 0·300 ms and so responses occurring within this latency range were accepted as originating from the neurone under study.

Figure 6 shows examples of the individual sweeps contributing to the average of the EPSP illustrated in Fig. 1. EPSPs occurring within the ±0·3 ms window can be

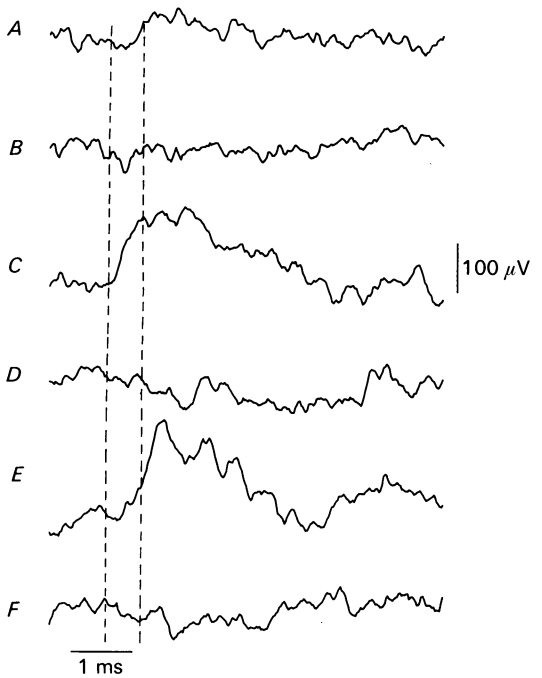

Fig. 6. Fluctuations in intracellular responses elicited by a temporalis spindle afferent in a masseter motoneurone (EPSP 6 in Table 1). The dashed lines enclose a window of points lying within ±0·3 ms of the start of the averaged EPSP (shown in Fig. 7*A*). Traces *A*, *C* and *E* show examples of EPSPs which commenced within this window and so were assumed to be triggered by the afferent under study. Traces *B*, *D* and *F* show examples of failures of transmission.

seen in traces *A*, *C* and *E* of Fig. 6 and these range in amplitude from approximately 70 μV (*A*) to 200 μV (*E*). Sweeps containing EPSPs were averaged in one buffer (and subsequently referred to as the extracted EPSP), and the sweeps not containing EPSPs (i.e. nils) averaged in another buffer. The results are shown in Fig. 7 for the unit illustrated in Fig. 6, together with the corresponding amplitude histogram plots. The extracted EPSP obtained for this unit was an order of magnitude larger than the averaged EPSP initially obtained by inclusion of all sweeps collected for the unit (9 μV in Fig. 7*A* and 110 μV in Fig. 7*B*). In contrast the nils yielded an averaged response which was essentially indistinguishable from the background variation in the baseline (Fig. 7*C*). This is better seen in Fig. 7*F* which shows the amplitude histogram obtained for the nils and the noise-only portion of the signal. A Student's *t* test on the two distributions confirmed the absence of any significant difference

($P = 0{\cdot}16$). In contrast the amplitude distribution of extracted EPSP amplitudes was significantly different from that of the noise (Fig. 7*E*; $P < 0{\cdot}001$). Similar findings were made for all other units tested.

The findings in Fig. 7 indicate an apparent incidence of failure of 87·2% for the connection, or alternatively that only 12·8% of the sweeps comprising the average

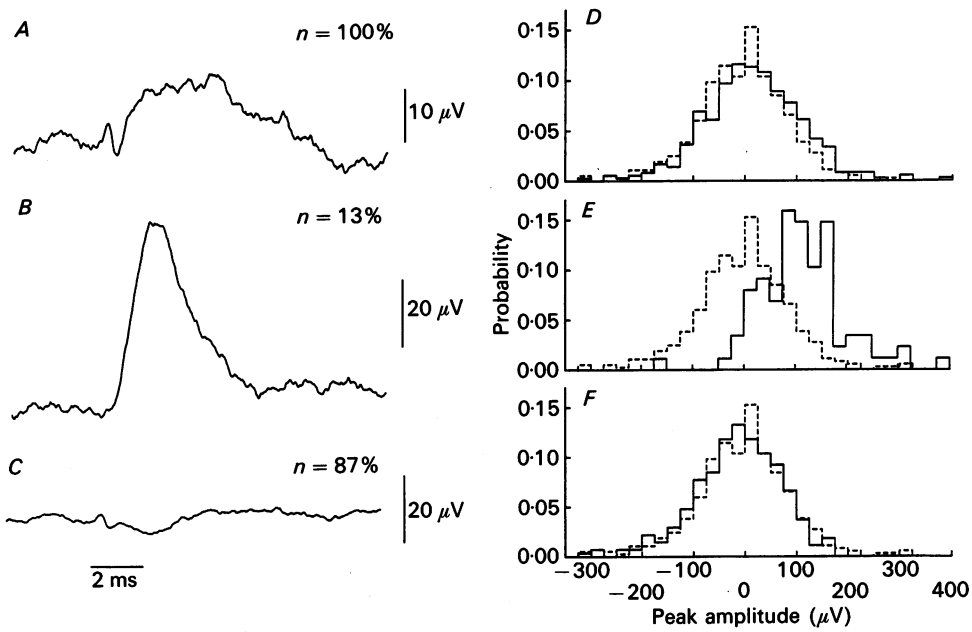

Fig. 7. *A–C*: *A* shows the averaged EPSP obtained by inclusion of all sweeps collected for the unit shown in Fig. 6; *B* shows the extracted EPSP obtained by averaging only those sweeps judged to contain an EPSP (see Fig. 6), and *C* shows the average obtained by inclusion of only sweeps with nil responses. Note the difference in amplitudes of the responses in *A* and *B* and the lack of an apparent response in *C*. *D–F*: amplitude histograms for the noise-only (dashed line), and noise + EPSP (continuous line) portions of the signal. *D* shows the distributions calculated for all sweeps collected ($n = 367$ sweeps); *E*, the distributions for the noise ($n = 367$) and extracted EPSPs ($n = 58$) averaged in *B*; *F*, the distributions of the noise ($n = 367$) and sweeps containing nils ($n = 309$).

contained EPSPs triggered by the presynaptic neurone. However, the result is open to the criticism that when very small numbers of sweeps are selected to produce a result, the result may be contaminated by EPSPs which may not belong to the presynaptic neurone being tested. The criticism can be answered by determining the incidence of randomly occurring EPSPs and then subtracting this value from the estimated incidence of occurrence of EPSPs in the window centred on the start of the averaged EPSP to produce a corrected incidence of occurrence of EPSPs. However, this correction does result in the apparent anomaly that even when an EPSP occurs on each sweep, subtraction of the incidence of randomly occurring EPSPs would produce a corrected incidence of less than 100%. The incidence of randomly occurring EPSPs was estimated within five arbitrarily selected ±0·3 ms windows

on each sweep of all perispike averages. Three of the windows were centred in the prespike period of the perispike average (usually at −6, −4 and −2 ms) and two in the postspike period (usually at 5 and 8 ms). The criterion adopted for acceptance of an estimate of corrected incidence of EPSPs was that the estimate of randomly

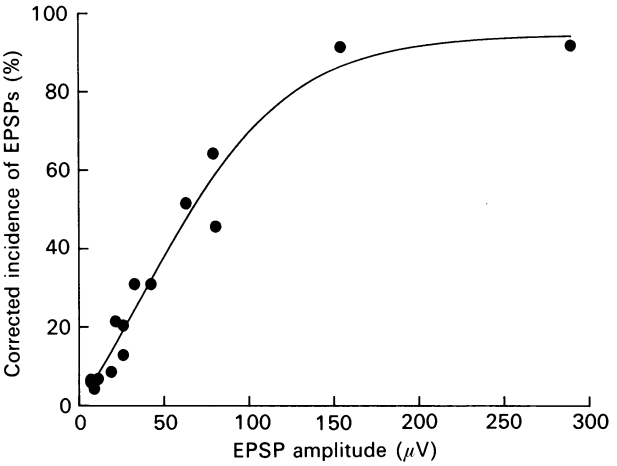

Fig. 8. Plot of the corrected incidence of EPSPs against amplitude of averaged EPSP obtained by inclusion of all sweeps ($r = 0{\cdot}98$).

occurring EPSPs in any one of the five windows should not exceed the estimated incidence of EPSPs in the window centred on the start of the averaged EPSP. For the sweeps used to construct the average in Fig. 7, the incidence of randomly occurring EPSPs in each of the five windows were 7·8, 7·6, 9·3, 7·8 and 10·1 %, giving a mean value of 8·5 %. The incidence of occurrence of EPSPs in the window centred about the start of the averaged EPSP was 12·8 %, giving a corrected estimate of 4·3 % (i.e. 12·8–8·5 %) for the incidence of occurrence of EPSPs which were most probably triggered by the presynaptic neurone under study.

A similar analysis was performed for all the EPSPs listed in Table 1. The mean incidence of randomly occurring EPSPs ranged from 3·1 to 11·6 % (mean = 5·8, S.D. = 2·3), and the amplitude of the extracted EPSPs ranged from 72 to 190 μV (mean = 118, S.D. = 32·9). The amplitude of the extracted EPSPs obtained by averaging just sweeps containing EPSPs triggered by an interneurone–spindle afferent ranged from 71 to 302 μV (mean = 132·7, S.D. = 60·3). The average amplitude of the extracted EPSP provides a measure of the size of the EPSPs which can be resolved with this method. The values obtained from randomly occurring EPSPs did not differ significantly from those obtained from the window centred on the start of the averaged EPSP ($P = 0{\cdot}3$; paired t test). The corrected incidence of occurrence of EPSPs triggered by the presynaptic neurone ranged from 4·3 to 92 %. There was a statistically significant ($r^2 = 0{\cdot}97$) relationship between the corrected incidence of occurrence of EPSPs and the amplitude of the averaged EPSP (Fig. 8), the relationship being described by a logistic sigmoid curve (i.e. log dose–response curve). Two points follow from this relationship. First, for averaged EPSPs of less than

approximately 150 μV amplitude, differences in amplitude of the averaged EPSPs elicited by presynaptic neurones can be accounted for primarily by differences in the incidence of failures of transmission. Thus, on the assumption of a minimum averaged EPSP amplitude of 150 μV and a steady firing rate of 100 impulses/s, interneurones could elicit a nominal steady depolarization of 3·6 mV and spindles a depolarization of 4·7 mV in elevator motoneurones (cf. values given above). Second, for EPSPs greater than 150 μV in amplitude, differences in the amplitude of EPSPs elicited by different presynaptic neurones may primarily be due to differences in either the number of active release sites or the quantal size. In this context it is intriguing to note that of the two EPSPs greater than 150 μV in amplitude, one is nearly twice the amplitude of the other (154 μV cf. 289 μV), hinting at the possibility of a systematic difference in either release sites and/or quantal size.

DISCUSSION

The results of this study provide a quantitative description of both the connectivity and the average amplitude of EPSPs elicited by single trigeminal interneurones and spindle afferents in elevator motoneurones. We have found that individual interneurones and spindle afferents may connect to similar numbers of elevator motoneurones and elicit EPSPs of similar amplitude. However, a high incidence of failures of transmission is a feature of transmission at the synapses of interneurones and spindle afferents onto motoneurones and this appears to be responsible for the relatively small averaged EPSP amplitudes elicited by interneurones and spindle afferents. The implications of these findings are discussed below.

There have been two previous reports on the connections of individual elevator spindle afferents onto elevator motoneurones (pentobarbitone anaesthetized cats: Appenteng *et al.* 1978; ketamine anaesthetized guinea-pigs: Nozaki, Iriki & Nakamura, 1985). Appenteng *et al.* (1978) obtained averaged EPSP amplitudes of 3·1–60 μV (mean = 18·3, S.D. = 15·4, n = 14), while Nozaki *et al.* (1985) reported values of 12–55 μV (mean = 21·5, n = 6). In the present study, only five of the fourteen EPSPs elicited by elevator spindle afferents had amplitudes of more than 60 μV and so there would appear to be a close similarity in the amplitudes of EPSPs elicited by elevator spindle afferents in the rat, cat and guinea-pig. Furthermore, we can extend the list to include EPSPs elicited by interneurones in rat elevator motoneurones. Thus the suggestion made by Appenteng *et al.* (1978) and Taylor & Gottlieb (1985) that interneurones may be capable of generating larger amplitude EPSPs in motoneurones than spindle afferents clearly does not apply to the specific group of interneurones examined in this study.

The question as to how the relative amplitude of single EPSPs elicited by spindle afferents compares to those elicited by other inputs is one that is receiving increasing attention. One of the earliest attempts to address this issue was made by Porter & Hore (1969) who compared minimal averaged EPSPs elicited in monkey forelimb motoneurones by electrical stimulation of corticospinal fibres and spindle primaries. Most corticomotoneuronal EPSPs were less than 200 μV, whereas most spindle primary EPSPs were greater than 200 μV in amplitude. However, the amplitude of the averaged synaptic responses obtained by signal averaging methods must be

interpreted with caution as they are dependent on the assumption that the postsynaptic response occurs following every impulse in the presynaptic neurone. Thus averaged responses, whether elicited by activation of single fibres or populations of fibres, can only safely be compared after taking into account any differences in failures of transmission. The problem has long been recognized but the difficulty has been in actually estimating the incidence of failures of transmission (e.g. see Watt, Stauffer, Taylor, Reinking & Stuart, 1976). Quantal analysis has so far provided the one generally accepted means of estimating both the magnitude and probability of occurrence of the underlying components of an EPSP (Jack *et al.* 1981; Harrison, Jack & Kullman, 1989; Kullman *et al.* 1989; Redman, 1990). However, for the reasons recently reviewed by Redman (1990), relatively few EPSPs can be successfully analysed in this way. Harrison *et al.* (1989) could find no qualitative or quantitative differences between single EPSPs elicited by graded electrical stimulation of fibres in the ventral quadrant, and of hindlimb Ia afferents. Both sets of EPSPs were found to be composed of between 1–5 quanta with a mean separation of approximately 100 μV (Jack *et al.* 1981; Harrison *et al.* 1989; Kullman *et al.* 1989). Components of zero amplitude (i.e. representing failures in transmission) have been relatively rare for those spinal EPSPs so far subjected to quantal analysis. For example, only 2/12 spindle primary EPSPs analysed by Jack *et al.* (1981) had components of zero amplitude and the probabilities associated with these were 18 and 22% (Table 2 of Jack *et al.* 1981). Our analysis suggests much higher incidences of transmission failure at synapses of interneurones and spindle afferents onto elevator motoneurones and so prompts questions as to why there should be such differences.

The difference may in part be more apparent than real and stem from a bias over the selection of spinal EPSPs so far analysed, a possibility specifically admitted by Jack *et al.* (1981). Cat triceps surae spindle primaries can elicit EPSPs of amplitudes ranging from 11–639 μV in homonymous motoneurones (pentobarbitone anaesthetized preparations: mean $\pm$ S.D. = 88·1 $\pm$ 5·8 μV, $n = 230$; Sypert, Fleshman & Munson, 1980) but the mean amplitude of the EPSPs so far subjected to quantal analysis have ranged from approximately 200 μV (Jack *et al.* 1981) to 300 μV (Harrison *et al.* 1989). Our data would lead to the suggestion that such large EPSPs are the very ones which are most unlikely to show significant failures of transmission and so in this respect the use of quantal analysis methods may have fostered the possibly erroneous belief that failures of transmission may be rare at the synapses of hindlimb spindle primaries onto hindlimb motoneurones. In this context the more interesting comparison would be between EPSPs elicited by trigeminal interneurones and spindle afferents in elevator motoneurones and those elicited by hindlimb spindle secondaries in homonymous motoneurones as the range of EPSP amplitudes is more comparable (3–136 μV; mean = 24·3 μV for presumed gastrocnemius spindle secondaries; cat: Sypert *et al.* 1980). However, spindle secondary EPSPs have not been subjected to either a quantal analysis or the sort of analysis performed in this study and so there is at present no basis for ascertaining if transmission at spindle secondary synapses on motoneurones is also characterized by a high incidence of failures. Such information is important as it bears on the question of whether the monosynaptic spindle secondary input onto spinal motoneurones may, like the spindle input onto elevator motoneurones, have also been underestimated.

The relatively high incidence of transmission failure at the excitatory synapses of trigeminal spindle afferents and interneurones onto elevator motoneurones could represent either an inherent feature of transmission at these synapses, or alternatively be the consequence of an inhibition of the synapses. We have preliminary evidence, based on use of the extracellular variant of the spike triggered averaging method, that transmission at the synapses of interneurones and spindle afferents onto elevator motoneurones can be enhanced two- to twelvefold following I.V. infusions of the NMDA antagonistic MK801 (Grimwood & Appenteng, 1991*a* and P. D. Grimwood, K. Appenteng & J. C. Curtis unpublished observations). Thus our working assumption has been that the high incidence of failures of transmission is the result of inhibition of the synapses but we have no specific evidence as to whether the inhibition operates at a pre- or postsynaptic level. Failures of transmission have generally been ascribed to presynaptic factors and the debate has been concerned with whether they stem from failure of the action potential to invade the terminals, or from a variability in the number of quanta released from individual boutons (Jack *et al.* 1981; Edwards, Harrison, Jack & Kullman, 1989; Redman, 1990). Our assumption is therefore that the transmission failures at synapses on elevator motoneurones may also have a presynaptic origin. However, pentobarbitone has been shown to reduce the amplitude of averaged EPSPs by means of presynaptic inhibition (Kullman *et al.* 1989) and so it could be argued that the high incidence of failures of transmission seen at the synapses on elevator motoneurones could be induced or augmented by the actions of the anaesthetic. This would imply that our findings may be of little physiological relevance. However, this can be discounted as similar incidences of failure have been obtained *in vitro* when studying the monosynaptic excitatory connections of neurones located around the region of the trigeminal motor nucleus onto presumed trigeminal motoneurones (J. C. Curtis, K. Appenteng & J. A. Moore, unpublished observations). This suggests that the high incidence of failures of transmission obtained in the present study are of physiological relevance and may be mediated by mechanisms, or pathways, either intrinsic to or local to the trigeminal motor nucleus. In this context, it may be significant that axo-axonic synapses are found on only 8% of all glutamate-immunoreactive boutons in the trigeminal motor nucleus (Saha, Appenteng & Batten, 1991). Glutamate-immunoreactive boutons form some 26% of all contacts in the trigeminal motor nucleus. If we assume that glutamate may be the major excitatory transmitter in the motor nucleus, then for most glutamate-immunoreactive boutons, any presynaptic control must involve either the operation of autoreceptors or the extrasynaptic release of transmitter.

Irrespective of what the precise mechanisms are, our data predicts that if there were no failures in transmission, all spindles and interneurones could elicit averaged EPSPs of 150 μV or more. This would suggest that these connections may be comparable, in terms of EPSP amplitude, to the spindle primary connections onto hindlimb motoneurones analysed by Jack *et al.* (1981) and Harrison *et al.* (1989). EPSP amplitude is determined by the interaction of a number of presynaptic and postsynaptic factors (see Burke, Fleshman & Segev, 1988; Nitzag, Segev & Yarom, 1990). Elevator motoneurones have been shown to be as electrically compact as spinal motoneurones, to have a similar complexity of branching (Moore & Appenteng,

1991), and a similar range of both input resistance and membrane time constant (Moore & Appenteng, 1990). Therefore, at least to a first approximation, the similarity in EPSP amplitudes could be taken to reflect common presynaptic factors, with one being the number of boutons given off to individual motoneurones. This would suggest that presynaptic neurones in different systems may give off an approximately similar number of contacts onto their target motoneurones or neurones. Thus one rule of organization may be that having identified a target motoneurone, individual presynaptic neurones then give off similar numbers of boutons onto their targets. The interest then would be in determining the factors limiting the numbers of boutons given off to individual target motoneurones and here one factor may be the motoneurone itself. However, an alternative argument is that similar amplitude EPSPs could still be observed even if there was a systematic difference in the numbers of boutons given off to individual elevator and spinal motoneurones because; (a) a portion of boutons belonging to one set of motoneurones may be systematically not active (silent), (b) there may be systematic differences in location of boutons along the motoneurone dendritic tree, or (c) there may be inherent differences in the properties of the boutons. Thus although the electrophysiology may hint at similarities in the organization of inputs onto elevator and spinal motoneurones, these could be produced by a number of different permutations which imply different strategies of organization of inputs onto motoneurones.

An implication of the above is that electrophysiological estimates of functional connectivity of neurones must be regarded with some caution as the values are dependent on the incidence of failures of transmission. This implies that connectivity, like EPSP amplitude, may also be a dynamically modulated parameter and modulations of both parameters may be one of the strategies routinely employed to modify 'set'. There is evidence that the functional connectivity of single hindlimb spindle primaries is increased immediately after spinalization (Nelson, Collatos, Niechaj & Mendell, 1979; Luscher, 1990), with some secondaries showing a near 70% increase in connectivity (Luscher, 1990). It is becoming increasingly evident that, even at synapses where the incidence of failures is low enough to allow the connection to be revealed by averaging, transmission onto motoneurones can still be further augmented by procedures such as changing the firing frequency of the presynaptic neurone (Collins, Honig & Mendell, 1984; Koerber & Mendell, 1991), or in the case of dorsal-spinocerebellar tract neurones, perfusing the central canal with a calcium rich perfusate (Walmsley & Nicol, 1991). Thus, connectivity is perhaps best estimated from a knowledge of both the number of boutons given off by a presynaptic neurone onto individual target neurones, and the total number of boutons given off to the population of neurones of interest. Without such morphological data it seems rather premature to attach too much importance to electrophysiological findings of limited connectivity as these may well not reflect the true capabilities of the system under study (e.g. see Appenteng *et al.* 1978; Kirkwood & Sears, 1982; Mendell 1984; Keirstead & Rose, 1988).

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